

# A case of hermaphroditism in the freshwater invading bivalve *Limnoperna fortunei* (Dunker, 1857) (Mytilidae) from Río de la Plata, Argentina

## Un caso de hermafroditismo en el bivalvo invasor *Limnoperna fortunei* (Dunker, 1857) (Mytilidae) en el Río de la Plata, Argentina

Gustavo A. DARRIGRAN\*, M. Cristina DAMBORENEA\* and Pablo E. PENCHASZADEH\*\*

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### ABSTRACT

The Southeast Asian freshwater mussel *Limnoperna fortunei* (Dunker, 1857) was first reported living in the Americas at Río de la Plata, Argentina, in 1991. Still earlier, it had invaded the Hong Kong area in the late '60s and Japan in the '70s. *L. fortunei* is dioecious, and no case of hermaphroditism have been previously reported. While studying the reproductive cycle of the Río de la Plata population of this species, three cases among 545 specimens studied were hermaphrodite representing only 0.55% of the total. In this contribution, a gonadal description of these cases of hermaphroditism in *L. fortunei* is presented. The histological study gave three kind of follicles, and their proportion in a hermaphrodite individual was 21% testis, 19% ovaries and 60% with both types of gametes.

### RESUMEN

*Limnoperna fortunei* (Dunker, 1857) es un bivalvo originario del sudeste asiático y se registró por primera vez para el continente americano en 1991 en el Río de la Plata, Argentina. A finales de la década del '60 había invadido el área de Hong Kong y Japón en la del 70. *L. fortunei* es una especie dioica, y no existe registro de casos de hermafroditismo. Durante el estudio del ciclo reproductivo de una población de esta especie en el Río de la Plata, se hallaron tres ejemplares hermafroditas entre 545 estudiados, representando sólo el 0,55% del total. En esta contribución, se presenta una descripción gonadal de los ejemplares hermafroditas. A través del estudio histológico se distinguen tres tipos de folículos y la proporción de éstos en el ejemplar hermafrodita es 21% testículos, 19% de ovarios y 60% de folículos con ambos tipos de gametas.

KEY WORDS: *Limnoperna fortunei*, hermaphroditism, invading bivalve, Neotropical region; Río de la Plata.

PALABRAS CLAVE: *Limnoperna fortunei*, hermafroditismo, bivalvo invasor, región Neotropical, Río de la Plata.

### INTRODUCTION

The Southeast Asian freshwater mussel *Limnoperna fortunei* (Dunker, 1857) was first reported living in the Americas at Río de la Plata in 1991 (PAS-

\*Depto. Científico Zoología Invertebrados, Museo de La Plata, Paseo del Bosque s/n (1900) La Plata, Argentina, CONICET. e-mail: darrigra@way.com.ar

\*\*Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina, CONICET.

TORINO, DARRIGRAN, MARTÍN AND LUNASCHI, 1993), where from a mean density of 5 individuals per sq m in 1991 it increased to 80.000 per sq m in 1993 (DARRIGRAN AND PASTORINO, 1995). Still earlier, it had invaded the Hong Kong area in the late '60s (MORTON, 1977) and Japan in the '70s (KIMURA, 1994). The Hong Kong population of *L. fortunei* is dioecious, and no case of hermaphroditism have been reported (MORTON, 1982, 1991). During a study on the reproductive cycle of the Río de la Plata population of this species, three cases of hermaphroditism were found. In this contribution, a description of these cases of hermaphroditism in *L. fortunei* is presented.

## MATERIAL AND METHODS

The hermaphrodite specimens (shell length 17.0, 0.9 and 14.0 mm) were sampled on February 28<sup>th</sup>, April 24<sup>th</sup> and September 28<sup>th</sup>, 1994, at Balneario Bagliardi (34° 55' S, 57° 49' W), Río de la Plata, Argentina, Neotropical region. The material was fixed in Zenker-formalin solution, dehydrated and embedded in paraffin; sections were cut at 7.5 microns, and stained with hematoxylin-eosin standards.

Sixty-six (66) microscope fields (100X) were examined, and the proportion of male and female follicles as well as the presence of both male and female gametes within a follicle were estimated. The maturation stages were recognized using LUBET'S (1959) scale. The present description is based on the February's specimen. Its body was divided into three regions: one anterior to the byssus gland, a middle region with byssus filaments and other posterior to the byssus.

The oocyte size of the hermaphrodite individual was compared with the sizes observed in the females of the same sample (length range from 11.0 to 18.0 mm), measuring the largest diameter of each oocyte with a distinct nucleolus; for this comparative purpose a Student t test was used.

## RESULTS

The gonad of *Limnoperna fortunei*, as in other Mytilidae, is very simple consisting of numerous diffuse follicles in the mantle and in the visceral mass. Three cases among 545 specimens studied were hermaphrodite, representing only the 0.55% of the total.

The proportion of the follicles in the hermaphrodite individual was 21% testis, 19% ovaries and 60% with both types of gametes. These three kind of follicles were present mainly in the mantle, filling between 30 and 40% of the tissue, the rest of which was connective tissue (Fig. 1). Only a low proportion of follicles was present in the visceral mass.

The 55% of male follicles were at a late developmental stage with full of mature spermatozoa, and with evidences of partial emission. In the other 45% of male follicles, no spermatozoa were distinguishable, and a proliferation stage was recognizable.

The 63% of female follicles were in a proliferation stage or partially spent. In the other 37% clear signs of necrosis of nonevacuated gametes and resorption were seen, especially on those in the visceral mass.

The presence of male and female cells in the same follicle, was the most common situation. In these follicles were male zones with maturing sperm and some with mature spermatozoa, as in the male follicles described above, as well as female zones showing different degrees of development, with a prevalence of young oocytes and some middle size oocytes attached to the follicle wall. In very few cases, oocytes bigger than 50 microns were observed, always free in the follicle lumen.

From 66 fields of vision, 53 of which present follicles with both male and female cells. Nevertheless, differences in the relative proportions of the three kinds of follicles were recognized in relation to the position in the animal (Fig. 2). In the anterior and middle region, and especially in the mantle, follicles with both male and female cells dominated (75 and 82% respectively), but in the pos-

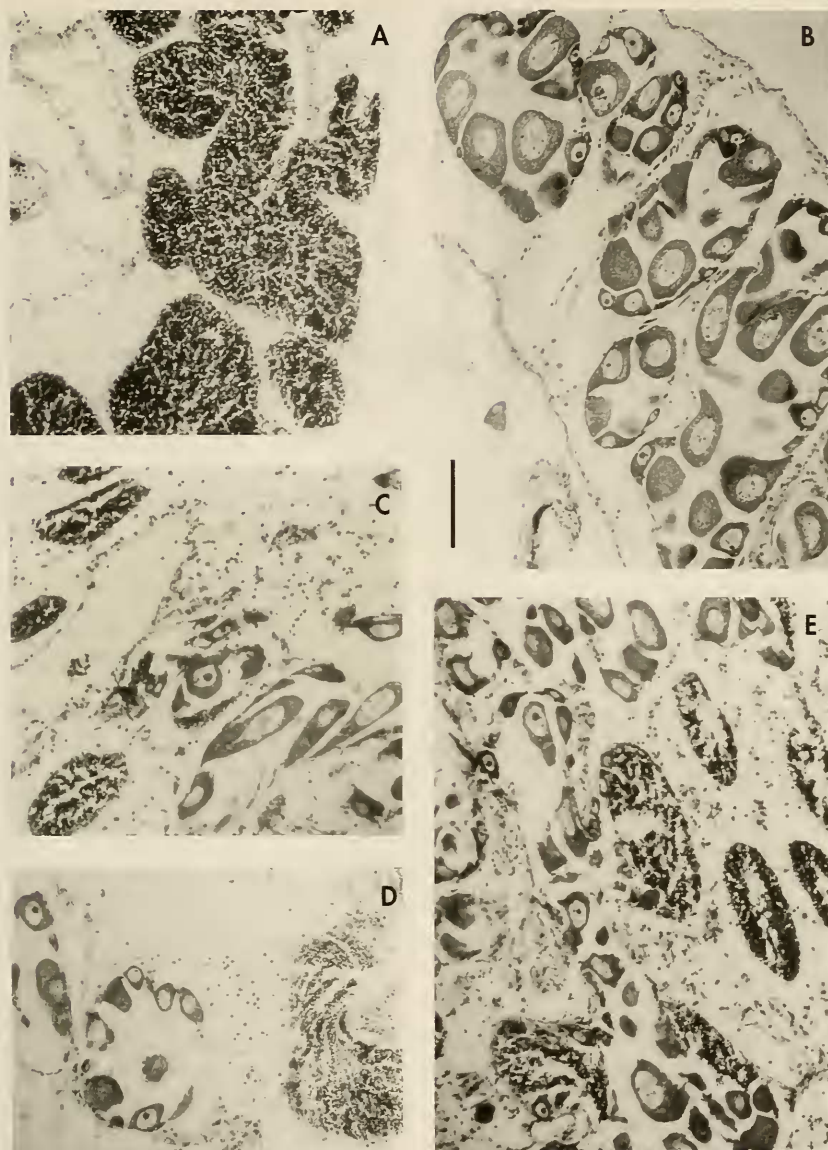


Figure 1. *Limnoperna fortunei* from Río de la Plata. Transverse sections of an adult male, an adult female and an hermaphrodite specimen; from a sample taken on the 28th Feb. 1994. A: well developed male with follicles filling the mantle tissue; B: well developed female with follicles filling the mantle tissue; C: hermaphrodite specimen showing a well developed female follicle and small male follicles; D: hermaphrodite specimen showing separate well developed male and female follicles; E: hermaphrodite specimens showing male and female gametes in the same follicles. Scale bars 100  $\mu$ m.

Figura 1. *Limnoperna fortunei* del Río de la Plata. Secciones transversales de un macho adulto, una hembra adulta y del ejemplar hermafrodita; de una muestra del 28 de febrero de 1994. A: macho maduro con folículos ocupando el tejido del manto; B: hembra madura con folículos ocupando el tejido del manto; C: espécimen hermafrodita con un folículo femenino bien desarrollado y folículos masculinos pequeños; D: espécimen hermafrodita mostrando folículos femeninos y masculinos separados y bien desarrollados; E: espécimen hermafrodita mostrando gametos femeninas y masculinas en el mismo folículo. Escalas 100  $\mu$ m.

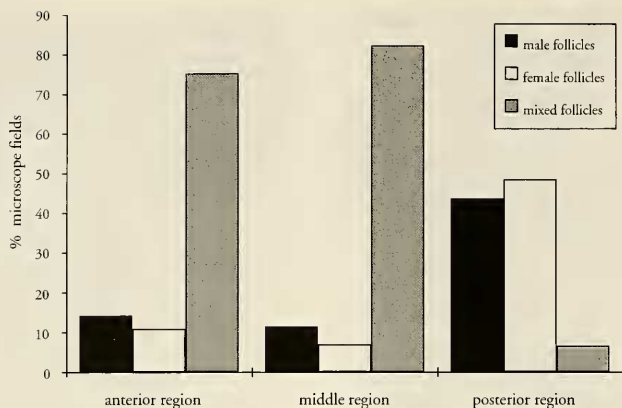


Figure 2. *Limnoperna fortunei* from Río de la Plata. Distribution of gonadic follicles in the hermaphrodite animal body.

Figura 2. *Limnoperna fortunei* del Río de la Plata. Distribución de los folículos gonadales en el cuerpo del animal hermafrodita.

terior part of the animal they represented only 8%, while the female follicles 49% and the male follicles 43%.

Adult males of the population from the same sample (Feb. 28<sup>th</sup>, 1994) are mature and had partially spent, however abundant cell proliferation is observed. The follicles filled most of the mantle tissue (70%). Adult females of the population, showed signals of partial emission, and in some cases, maturing oocytes, the follicles occupy 60-70% of the mantle tissue. The observed mode of the oocyte size was larger in the normal animals in comparison to the hermaphrodite one (Fig. 3). In the hermaphrodite specimen, the largest oocytes were found in the exclusively female follicles (mean diameter of 47 microns, SD= 19.2, n= 154) while the oocytes in the mixed follicles were much smaller (mean diameter 27 microns, SD= 11.1; n= 227). The mean oocyte diameter in both kinds of follicles was significantly different ( $F= 3.0073$ ; d. f. = 177;  $P < 0.001$ ).

## DISCUSSION

*Limnoperna fortunei* is a dioecious species in its habitat at Hong Kong area (MORTON, 1982) and in its newly coloni-

zed environment in the Río de la Plata. This report of hermaphroditism is the first one for the species.

The observed percentage of hermaphroditism is about the same found in the other generally dioecious species of bivalves, i. e. *Placopecten magellanicus* (Gmelin, 1791) 1.3% (NAIDU, 1970); *Crasostrea virginica* (Gmelin, 1791) 0.4% (KENNEDY, 1983); *Anadara granosa* (Linnaeus, 1758) 0.3% (Broom, 1983); *Perna viridis* (Linnaeus, 1758) 0.1% (Lee, 1988); *Mytilus edulis* (Linnaeus, 1758) 0.1% (SUNILA, 1981).

The presence of intermingled male and female follicles is one, among other arrangement patterns, recorded in hermaphrodite bivalve species, i. e. in *Laevicardium laevigatum* (Linnaeus, 1758) where male follicles first developed, ovarian follicles appear from peripheral points of the testicular follicles, resulting in a simultaneous hermaphrodite (PENCHASZADEH AND SALAYA, 1983). In the case reported here, three kinds of intermingled follicles were found: male follicles, female follicles and follicles with both male and female gametes. Most of the male follicles and female follicles were already partially spent. Mixed follicles, on the contrary, showed a proliferation of male cells. The development stage in the male follicles and female follicles of

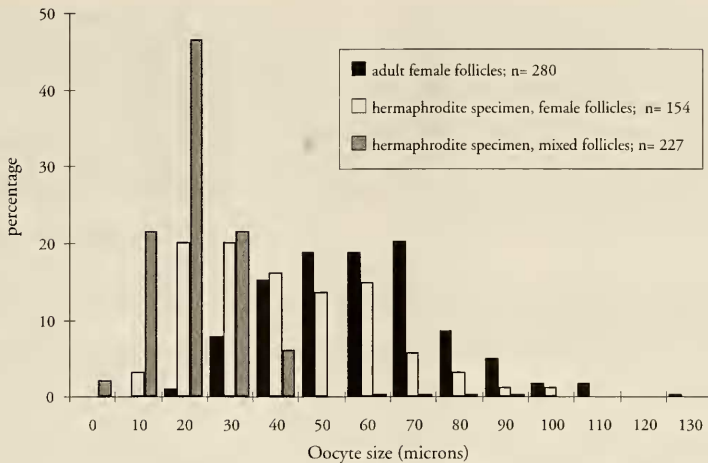


Figure 3. *Limnoperna fortunei* from Río de la Plata. Oocyte size frequency distribution in the hermaphrodite specimen compared with oocytes in adult females sampled at the same date (Feb. 28<sup>th</sup>, 1994).

Figura 3. *Limnoperna fortunei* del Río de la Plata. Distribución de frecuencias de la talla oocitaria del ejemplar hermafrodita comparado con la de las hembras adultas de igual fecha (28 Feb. 1994).

the hermaphrodite, is coincident in general terms, with the observations made on the normal dioecious individuals of the same population sample. Nevertheless the mantle occupation by the follicles is lower in the hermaphrodite, as well as the oocyte diameter mean, especially those from mixed follicles.

Many freshwater species of bivalves are hermaphroditic and brooding (i. e. Sphaeriidae, some Corbiculidae) but there are some species which show wide intraspecific variability in sexual expression, as *Corbicula fluminea* (Müller, 1774) in the Holarctic; others, as *Anodonta woodiana* (Lea, 1863) (Unionidae) and *Limnoperna fortunei* (Mytilidae) are dioecious (MORTON, 1991).

The dioecious condition is the main fact in Mytilidae, which includes mostly marine and estuarine species (LUBET, 1959; LUNETTA, 1969; NASCIMENTO, 1971;

LEE, 1988); and *Limnoperna fortunei* is not an exception among them.

The observation of both male and females gametes present in the same follicle is similar to the observations made in unusual hermaphrodites of *Crassostrea glomerata* (DINAMANI, 1974) and *Pecten maximus* (MASON, 1958), and is a rare phenomena in the protandric hermaphrodite *Laevicardium laevigatum* (PENCHASZADEH AND SALAYA, 1983).

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